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(54) **Title:** METHOD OF TREATING OR PREVENTING OBEISITY AND LIPID METABOLISM DISORDERS AND COMPOSITIONS FOR USE THEREIN

(57) **Abstract:** The present invention provides a method of preventing or treating human obesity, said method comprising ingesting a composition containing, calculated on dry matter: 10-100 wt.% protein hydrolysate; 0-90 wt.% intact protein; 0-50 wt.% carbohydrate; and wherein hydrolysed protein and intact protein together are present in a concentration (w/w) that exceeds the carbohydrate concentration (w/w). The invention also encompasses the use of the same composition in a method of preventing or treating lipid metabolism disorders and in a method for improving body appearance. Other aspects of the invention relate to nutritional beverages, snacks and soups that can advantageously be employed in accordance with the aforementioned methods.

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METHOD OF TREATING OR PREVENTING OBESITY AND LIPID METABOLISM DISORDERS AND COMPOSITIONS FOR USE THEREIN

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a method for preventing or treating obesity as well as for preventing or treating lipid metabolism disorders. Also provided herein is a cosmetic method for improving body appearance.

In addition, the present invention concerns compositions for use in the aforementioned methods. More particularly, the invention provides nutritional beverages, snacks and soups for use in said methods.

BACKGROUND OF THE INVENTION

Obesity is a risk factor associated with various adult diseases such as diabetes type 2 and cardiovascular diseases including hypertension. It may also be a cause for deteriorating these diseases. Both adults and children may face the problem of obesity. Currently, about 25% of the children and 50% of the adults in the USA are overweight, prevalence rates that have increased by 50% since the 1960s. The obesity in children has been associated with consumption of sugar-sweetened drinks.

Lipid metabolism disorders are a well-known complication of obesity. These disorders are often characterised by hyperinsulinaemia, elevated apolipoprotein B levels, high triglycerides concentration, the presence of small dense LDL, high LDL cholesterol concentration, and low HDL cholesterol concentration.

With the trend of healthy eating, also the trend of being fit or stay lean has emerged. Accordingly, not only persons affected by obesity are concerned with the problem of controlling or losing weight, but also healthy individuals, individuals who do not suffer from obesity often have a desire to stay lean or to lose weight in order to obtain a cosmetically more acceptable or more desirable appearance.

The growing concern of the human population about overweight has resulted in an increasing intensity of research in this field. Recently, Jequier concluded ("Pathways to obesity" Int J Obes Relat Metab Disord 2002; 26 (suppl):2:S12-7) that the prevalence of obesity had reached epidemic proportions in affluent societies and indicated that the primary cause of obesity was lying more in environmental and behavioural changes than in genetic modifications.

It has been tried to solve the problem of overweight by using of medication. This approach, however, suffers from the disadvantage that after a period of about 3 months patients seem to reach a plateau as far as weight loss is concerned. Also, the medication does not diminish the "hungry" feeling, i.e. it fails to impart satiety. Another disadvantage of the use of medication is the potential for side effects, which sometimes outweighs the benefits of taking medication.

Accordingly, studies were done to find a more natural solution. One simple natural solution is to exercise. However, to obtain a noticeable result, exercising on a regular basis is a pre-requisite that, with today's trends of habits, is not always feasible. Another simple natural solution is to eat less or to adapt the diet. Remedies based on consumption of low-fat diets have been found to produce some result. On the short-term low-fat diets induce a modest weight loss in obese individuals but on the longer term (more than a year) such diets appear to have little effect on body fatness.

Many low fat commercial food products are based on the recommendations of the American Hearth Association (AHA) and derive a low percentage of energy from fat and a high percentage from carbohydrate. Despite the popularity of these products and the reduction (on population base) of the percentage of dietary energy derived from fat, the prevalence of obesity has continued to increase. This clearly suggests that fat is only one of several determinants and not the primary cause of the high prevalence of excess body fat in our Western society. ("Is dietary fat a major determinant of body fat?", Am. J. Clin. Nutr. 1998; 67(suppl):556S-62S.).

Another dietary factor that may play a critical role in body weight regulation is the glycaemic index (GI). The concept of the GI was proposed by Jenkins et all ("Glycemic index of foods: a physiological basis for carbohydrate exchange". Am J Clin Nutr 1981;34:362–366), to characterize the rate of carbohydrate absorption after a meal. It is defined as the area under the

glucose response curve after consumption of 50 g carbohydrate from a test food divided by the area under the curve after consumption of 50 g carbohydrate from a control food, either white bread or glucose. Many factors together, including carbohydrate type, fibre, protein, fat, food form and method of preparation, determine the GI of a particular food.

Recently a new method of dieting called the "Montignac method" has been described. This method involves balancing the administration of protein and carbohydrate. In particular, the Montignac method is an *ad libitum* low GI/low fat/high protein diet in which carbohydrates with a glycaemic index above 55 are excluded.

In the underlying physiological mechanism insulin is believed to play an important role. Insulin stimulates the uptake of glucose from the blood and subsequent conversion of glucose into the storage carbohydrate glycogen or into lipids when glycogen storage is saturated.

It is well known that amino acids can stimulate the release of insulin upon a glucose load. A rapid rise of the glucose level in the blood induces a quick increase in the insulin level, resulting in a rapid fall of the blood glucose level. This induces a feeling of craving or hunger and urges someone to eat. It is speculated that this phenomenon plays an important role in the weight gain observed in diets based on low fat and high carbohydrates.

Although the Montignac method has shown good results, people have found it difficult to follow it on a long-term basis, especially once the weight loss is obtained. As a result, the benefits obtained are often lost within a few weeks after discontinuation of the method.

SUMMARY OF THE INVENTION

It has now been found that repeated ingestion of a composition containing at least 10% protein hydrolysate from a non-mammalian source by dry weight, provides a natural solution to the problem of losing weight as well as to the problem of lipid metabolism. This effect is especially seen upon long term use of the composition. The present composition is characterised in that it optionally contains intact protein and/or carbohydrate, provided hydrolysed protein and intact protein together are present in a concentration (w/w) that exceeds the carbohydrate concentration (w/w). The presence of high levels of carbohydrate, relative to the total content of non-mammalian hydrolysed protein and intact protein, is likely

to produce an undesired insulin response that will counteract the desired impact of the present method.

In one aspect of the invention, there is provided a method for preventing and/or treating human obesity, said method comprising ingesting a composition, containing, calculated on dry matter:

10-100 wt% protein hydrolysate;
0-90 wt.% intact protein;
0-50 wt.% carbohydrate; and

wherein hydrolysed protein and intact protein together are present in a concentration (w/w) that exceeds the carbohydrate concentration (w/w).

In another aspect of the invention, there is provided a method of preventing or treating lipid metabolism disorders, said method comprising ingesting the compositions as defined above.

In another aspect of the invention, there is provided a cosmetic method for stimulating and/or improving body weight reduction in order to improve the human body appearance, said method omprising ingesting the composition as defined herein before.

A further aspect of the invention relates to a nutritional beverage containing:
0.5-10 wt.% hydrolysed protein from a non-mammalian source;
0-10 wt.% intact protein;
0.5-5 wt.% carbohydrates;
artificial sweetener;
flavouring; and
at least 80 wt.% water;
wherein hydrolysed protein and intact protein together are present in a concentration (w/w) that exceeds the carbohydrate concentration (w/w).

Yet another aspect of the invention concerns a savoury snack product containing:
3-50 wt.% hydrolysed protein from a non-mammalian source;
10-50 wt.% intact protein;
0-50 wt.% carbohydrate;
0.3-3 wt.% salt; and

0.1-20 wt.% water;
wherein hydrolysed protein and intact protein together are present in a concentration (w/w) that exceeds the carbohydrate concentration (w/w).

Finally, the present invention also encompasses a soup containing:
0.5-20 wt.% hydrolysed protein from a non-mammalian source;
0.5-30 wt.% intact protein;
0-20 wt.% carbohydrate;
0.1-2 wt.% salt; and
at least 70 wt.% water;
wherein hydrolysed protein and intact protein together are present in a concentration (w/w) that exceeds the carbohydrate concentration (w/w).

DETAILED DESCRIPTION OF THE INVENTION

A - protein hydrolysate

The use of a protein hydrolysate from a non-mammalian source is an essential element of the present invention. Indeed, the use of a composition containing at least 10% of this ingredient by weight of dry matter has been found beneficial for patients suffering from obesity, but also for healthy persons wanting to stay lean. Accordingly, the use of such a protein hydrolysate has been found effective in the prevention and treatment of human obesity. Advantageously, it was also found to be effective in the prevention and treatment of lipid metabolism disorders in humans, in particular by reducing LDL cholesterol concentration and/or reducing the triglycerides concentration and/or increasing HDL cholesterol concentration. Preferably, such treatment results in an improvement of all three parameters.

Yet, another group of persons that can derive benefit from ingesting protein hydrolysates from a non-mammalian source are healthy individuals who have a desire to stay lean. The term "healthy individuals" as used in here refers to individuals not suffering from obesity.

The term "non-mammalian proteins" refers to proteins that are not obtained from mammalian sources. Accordingly, non-mammalian proteins include proteins from the non-limiting

sources: vegetable proteins, fungal proteins, microbial proteins, fish proteins, poultry proteins, egg proteins, as well as mixtures thereof.

The term "protein hydrolysate" is used to define a protein raw material that has been hydrolysed by one or more hydrolytic enzymes. The hydrolytic enzyme can be of animal, plant, yeast, bacterial or fungal origin. Preferably enzyme preparations are used which have a low exo-peptidase activity to minimise the liberation of free amino acids and to improve taste profiles of the protein hydrolysates. The preferred hydrolysed protein material of the present invention has an average peptide chain length in the range of 2-40 amino acid residues and more preferably in the range of 3-20 amino acid residues. The average peptide chain can be determined using the method as described in WO 96/26266. The protein hydrolysates that can be used to prepare a composition as disclosed in the present invention are not limited to ones disclosed in the present invention but include all protein hydrolysates that can be obtained by enzymatic hydrolysis using common techniques as described in the literature and known to those skilled in the art.

Preferably, the non-mammalian protein hydrolysates used in accordance with the present invention are obtained from a protein selected from vegetable proteins, fungal proteins, microbial proteins, fish proteins, poultry proteins, egg proteins, and mixtures thereof.

Vegetable protein hydrolysates are hydrolysates of vegetable protein obtained from protein selected from wheat, maize, pea, rice, soy, barley, oats, potato, and mixtures thereof, more preferably the vegetable protein hydrolysate is a pea protein hydrolysate, a rice protein hydrolysate or a soy protein hydrolysate. Most preferably, the hydrolysate is a pea protein hydrolysate.

For the purpose of the present invention, the vegetable protein hydrolysates are preferred. Indeed, it has been found that the vegetable protein hydrolysate, especially the hydrolysates obtained from pea protein, rice protein and/or soy protein, best influenced the weight reduction in obese people on a hypocaloric diet.

Ingestion of a composition containing at least 10% of the protein hydrolysate of a non-mammalian source by weight of dry matter induces a long lasting feeling of satiety (which stops people from eating even in *ad libitum* situations) without giving rise to a substantial

increase in the blood insulin level (which urges people to eat), even if the composition also contains a significant amount of carbohydrate. This is related to the high solubility of the present protein hydrolysate, which allows it to reach the intestinal tract quickly and in a relatively high concentration. It was observed that, even in situations where food is available *ad libitum*, individuals consume less in the hours after ingesting the present composition containing the non-mammalian protein hydrolysate. This obviously has positive implications for the ability to control body weight.

The non-mammalian protein hydrolysate is preferably incorporated in a composition comprising one or more materials selected from the group consisting of carbohydrates, intact proteins (both mammalian and non-mammalian), free amino acids, minerals, vitamins, dietary fibers, herbals, spices, flavors, fat, and mixtures thereof. Preferably, the composition comprises a carbohydrate, more preferably together with materials selected from the group consisting of intact proteins, free amino acids, minerals, vitamins, dietary fibers, herbals, spices, flavors, fat, and mixtures thereof.

B – Methods of preventing or treating obesity or lipid metabolism disorders

The present methods employ a composition that may optionally contain carbohydrate, provided the amount of carbohydrate does not exceed the combined amount of non-mammalian protein hydrolysate and intact protein contained within the same composition. In a particularly preferred embodiment, the present composition contains proteinaceous matter and carbohydrate in a weight ratio within the range of 1.5:1 to 9:1, most preferably within the range of 1.5:1 to 4:1. For the purpose of this ratio calculation, the term "proteinaceous matter" includes the present protein hydrolysate from a non-mammalian source as well as free amino acids and intact protein material.

The term "intact protein" refers to proteins that have not been subjected to modifications such as hydrolysis. The term "free amino acid" refers to amino acids *per se*, i.e. said term does not include amino acid residues contained in indirect sources like proteins.

The benefits of the present composition are particularly pronounced in case the composition contains the protein hydrolysate from a non-mammalian source in a higher amount than the

optional carbohydrate component. In a particularly preferred embodiment, the composition contains the protein hydrolysate and the optional carbohydrate in a weight ratio of more than 1:3, more preferably in a weight ratio of more than 1:1.

The present composition preferably does not contain substantial amounts of carbohydrate as excess amounts of carbohydrate may lead to undesirable insulin surges, despite the tempering effect of the non-mammalian protein hydrolysate. In a preferred embodiment, the composition contains 0-30% carbohydrate by weight of dry matter.

The present composition advantageously contains intact protein. Intact protein is suitably incorporated as an energy source and to improve the palatability of the composition. Typically, the composition contains 20-80% intact protein by weight of dry matter.

The present composition may suitably contain some carbohydrate material. Indeed, by selecting the right type(s) of carbohydrate, it was found that the insulin response, believed to be an important factor in weight gain, was neglectable, even after a few weeks of dieting with the present composition.

On the basis of their rate of release of glucose into the blood, carbohydrates can be divided into three classes (Cummings JH and Englyst HN, AJCN 1995;61 (4 Suppl):938S-945S). The carbohydrate of the invention is advantageously selected from at least one member of these classes:

a) rapidly absorbed carbohydrates.

This class comprises carbohydrates such as glucose and disaccharides containing a glucose unit such as sucrose, lactose, maltose and galactose that contribute directly to elevation in blood glucose. Sucrose, in addition to being rapidly absorbed, has the advantage of imparting a sweet taste to the composition thereby increasing palatability.

To keep the insulin response low after consumption of these type of carbohydrates it is preferred to limit the intake to not more than 6, preferably not more than 5 gram per serving or per moment of eating.

To limit the intake of the rapidly absorbed carbohydrates to 6 gram for a beverage with a typical serving of 200 ml, the concentration therefore should not exceed 6 gram per 200 ml (3% w/v). In beverages for which the typical serving is larger the concentration should be

accordingly lower. A snack, such as a nutritional bar, typically weighs 60 grams. In order to limit the intake of the rapidly absorbed carbohydrates in such a snack to not more than 6 gram, the concentration should not exceed 6 gram per 60 gram (10% w/w).

b) moderately absorbed carbohydrates

This class comprise mono- and disaccharides, that do not contribute directly to elevation of blood glucose as well as those soluble and insoluble polysaccharides (e.g. starches) that contain at least 30 molar % glucose units and that release a majority of this glucose upon incubation in pancreatic amylase and amyloglucosidase at 37°C in 20 minutes or less.

The monosaccharides and disaccharides that are considered moderately absorbed are non-glucose monosaccharides and non-glucose-containing disaccharides that contribute to blood glucose levels indirectly, i.e., after a metabolic event occurs, e.g., conversion into glucose by the liver. Non-limiting examples of such moderately absorbed carbohydrates are mannose and fructose. The class of moderately absorbed carbohydrates also includes polysaccharides that contain glucose units and have a dextrose equivalent of 15 or lower, such as white flour, wheat flour, certain starches, and the like.

To keep the insulin response low after consumption of these type of carbohydrates it is preferred to limit the intake to not more than 20 gram per serving or per moment of eating.

c) slowly absorbed carbohydrates

This class comprises polysaccharides (carbohydrates containing three or more monomeric units) that contain at least 30 molar % glucose units, have a glycaemic index greater than 2, and release a majority of their glucose in greater than 20 minutes upon incubation in pancreatic amylase and amyloglucosidase at 37°C. Non-limiting examples of slowly absorbed polysaccharides include corn starch, high amylose corn starch (e.g. corn starch with an amylose content of greater than 40% by weight) and modified starches with a glycaemic index less than 80, preferably of less than 60. Examples of such products are Novelose, Systain 550 and 735 from National Starch.

Accordingly, the carbohydrate of the invention is advantageously selected from at least one member of the class consisting of:

a) a rapidly absorbed fraction comprising components selected from glucose, sucrose, one or more rapidly absorbed disaccharides containing a glucose unit, and mixtures thereof;

b) a moderately absorbed fraction comprising components selected from one or more moderately absorbed monosaccharides, disaccharides, glucose-containing polysaccharides, and mixtures thereof;

c) a slowly absorbed fraction comprising components selected from one or more slowly absorbed glucose-containing polysaccharides.

The carbohydrates incorporated in the present composition have to be selected carefully in order to ensure that ingestion of the composition will not be accompanied by major insuline surges in the blood. Preferably, to this end, at least a sizeable fraction of the carbohydrates should be slowly released and/or slowly digested such that the release of glucose into the blood is slow.

In case the present composition contains carbohydrate, it is preferred that at least a fraction of said carbohydrate is slowly absorbed carbohydrate. The slowly absorbed fraction of the carbohydrate component typically constitutes about 1 to about 95 weight (wt) % of total carbohydrate component, preferably about 5 to about 85 wt %, and more preferably about 20 to about 75 wt %.

The present composition may suitably contains rapidly and/or moderately absorbed carbohydrates. Preferably, the composition contains between 0.5 and 5% rapidly and/or moderately absorbed carbohydrates by weight of dry matter. The rapidly absorbed fraction of the carbohydrate component typically constitutes about 1 to about 95 weight (wt) % of total carbohydrate component, preferably about 5 to about 85 wt %, and more preferably about 20 to about 75 wt %. The moderately absorbed fraction of the carbohydrate component typically constitutes about 1 to about 95 weight (wt) % of total carbohydrate component, preferably about 5 to about 85 wt %, and more preferably about 20 to about 75 wt %.

In a particularly preferred embodiment, a major part of the carbohydrates contained in the present composition are slowly absorbed carbohydrates. Even more preferably, the composition preferably contains slowly absorbed carbohydrates in a concentration (w/w) that exceeds the combined concentration (w/w) of rapidly and moderately absorbed carbohydrates by at least a factor 2.

Preferred carbohydrates are carbohydrates wherein the rapidly absorbed fraction is selected from glucose, sucrose, maltose, and mixtures thereof, the moderately absorbed fraction is selected from fructose, mannose, maltodextrin, white flour, wheat flour, and mixtures thereof, and the slowly absorbed fraction is selected from raw corn starch, high amylose corn starch, modified starch, and mixtures thereof.

For solid or semi-solid compositions the slowly absorbed polysaccharide preferably comprises high amylose corn starch, modified starch (as described above), or a mixture thereof. A preferred slowly absorbed carbohydrate is Novelose resistant starch, which is a high amylose corn starch available from National Starch. For liquid products, raw corn starch is preferred.

The composition of the invention optionally contains one or more flavours including natural or artificial flavourants to enhance palatability such as herbals, spices, flavour ingredient. Any flavourant used in the art can be included such as strawberry; cherry; chocolate; orange; citrus; lemon; grapefruit; coconut; vanilla; spices such as nutmeg, cinnamon and the like.

The composition may suitably contain a fat component. The fat component can be any lipid or fat known in the art to be suitable for use in nutritional compositions. Typical fats include milk fat, safflower oil, canola oil, egg yolk lipid, olive oil, cotton seed oil, coconut oil, palm oil, palm kernel oil, soybean oil, sunflower oil, fish oil and fractions of all above oils derived thereof such as palm olein, medium chain triglycerides (MCT), and esters of fatty acids wherein the fatty acids are, for example, arachidonic acid, linoleic acid, palmitic acid, stearic acid, docosahexaeonic acid, eicosapentaenoic acid, linolenic acid, oleic acid, lauric acid, capric acid, caprylic acid, caproic acid, and the like. High oleic forms of various oils are also contemplated to be useful herein such as high oleic sunflower oil and high oleic safflower oil. Preferably the fat level in the composition is such that less than 40% of the energy in the composition is provided by fat.

The composition employed in the present methods may suitably be free from carbohydrates, especially if said product is intended to be used as a nutraceutical. Alternatively, said composition may be a food product that meets the compositional requirements described herein before. Such food products, in addition to the present protein hydrolysate, typically will contain intact protein, carbohydrates and optionally fat. Modified food products that are

particularly suited for use in the present method include beverages, snacks and soups. The latter products are suitably consumed between meals. Thus, by ingesting such a product between 0.5 and 2 hours prior to the next meal, a feeling of satiety will be induced that will persist throughout the meal, leading to less caloric intake.

The benefits of the present method will become particularly apparent in case the method is continued uninterruptedly for at least 2 weeks, more preferably at least 4 weeks, most preferably at least 3 months. Typically, the method according to the invention comprises at least once daily ingestion of the present composition. More preferably, said composition is ingested at least twice daily.

Typically, the present method comprises ingesting the present composition in an amount equivalent to at least 5 g, preferably at least 10 g of the combination of protein hydrolysate and intact protein per serving. Most preferably, the aforementioned minimum amounts relate to the amount of non-mammalian protein hydrolysates that are ingested per serving.

C) – Products suitable for use in the present method

The method described herein before may suitably employ a composition in the form of a food products, especially a food product selected from a nutritional beverage, a snack and a soup.

Accordingly, one aspect of the present invention relates to a nutritional beverage containing:

- 0.5-10 wt.%, preferably 0.8-6 wt.%, more preferably 1-4 wt.% hydrolysed protein from a non-mammalian source;
- 0-10 wt.%, preferably 1-9 wt.% intact protein;
- 0.5-5 wt.% carbohydrates, preferably rapidly absorbed carbohydrates;
- artificial sweetener;
- flavouring; and
- at least 80 wt.% water;

wherein hydrolysed protein and intact protein together are present in a concentration (w/w) that exceeds the carbohydrate concentration (w/w).

The aforementioned beverage is particularly suited for use in the present method because it can be consumed conveniently at any moment during the day. Also this embodiment offers the advantage that it is feasible to formulate a pleasant tasting low caloric beverage that can suitably be used to deliver a large amount of protein hydrolysate. In a preferred embodiment, hydrolysed protein and intact protein together represent at least 5 wt.% of the beverage.

Another aspect of the invention is concerned with a savoury snack product containing:

- 3-50 wt.%, preferably 4-30 wt.%, more preferably 5-20 wt.% hydrolysed protein from a non-mammalian source;
- 10-80 wt.%, preferably 20-70 wt.% intact protein;
- 0-50 wt.% carbohydrate; preferably 0-30 wt.% carbohydrate;
- 0.3-3 wt.% salt; and
- 0.1-20 wt.% water;

wherein hydrolysed protein and intact protein together are present in a concentration (w/w) that exceeds the carbohydrate concentration (w/w).

The present snack product may suitably take the form of a nutritional bar or a baked or fried product. In a particularly preferred embodiment, the snack product contains 1-20 wt.% dietary fibre.

Yet another aspect of the invention relates to a soup containing:

- 0.5-20 wt.%, preferably 1-20 wt.%, more preferably 2-20 wt.% hydrolysed protein from a non-mammalian source;
- 0.5-29 wt.%, preferably 1-25 wt.% intact protein;
- 0-20 wt.% carbohydrate;
- 0.1-2 wt.% salt; and
- at least 70 wt.% water;

wherein hydrolysed protein and intact protein together are present in a concentration (w/w) that exceeds the carbohydrate concentration (w/w).

The composition and products according to the present invention may suitably comprise one or more optional ingredients selected from intact proteins, free amino acids, minerals, vitamins, dietary fibers, herbals, spices, flavors, fat, and mixtures thereof. Typical examples of minerals, vitamins and other nutrients that may be employed in the present composition and products include vitamin A, vitamin B6, vitamin B12, vitamin E, vitamin K, vitamin C,

vitamin D, inositol, taurine, folic acid, thiamine, riboflavin, niacin, biotin, pantothenic acid, choline, calcium, phosphorous, iodine, iron, magnesium, copper, zinc, manganese, chloride, potassium, sodium, carotenoids, flavonoids, lipoic acid, nucleotides, selenium, chromium, molybdenum, and L-carnitine. Minerals are usually added in salt form.

Typical dietary fibers include fibers and non-absorbant carbohydrates that have a glycaemic index less than 2. The fiber can be soluble, insoluble, fermentable, non-fermentable, or any combination thereof. The fiber can be, for example, soy fiber, pectin, certain resistant starches, oligofructose, inulins, oat fiber, pea fiber, guar gum, gum acacia, modified cellulose, and the like.

D) - Examples

The present invention is illustrated in the non-limiting following examples:

Example 1. Spiced gingerbread

The following is the composition of a spiced gingerbread incorporating the invention, which ingredients were mixed into a homogeneous batter prior to being baked in an oven for 75 min at 150 °C. The list of ingredient is as follows:

50 gram wheat gluten
125 gram wheat starch
45 gram fructose
150 gram Systain® 735 (National Starch)
15 gram biscuit spices
10 gram cinnamon
300 ml sugar free fruit syrup
15 gram baking powder
400 ml water
375 gram pea protein hydrolysate Hyprol™ 7102 (Quest International)
300 gram apple pieces

Example 2. Soda drink

A protein-rich drink was prepared by adding 9.16 grams per 100 ml of the powder composed out of the following ingredients:

Hydrolysed pea protein (agglomerated Hyprol™ 7102 Dev)	7.50 g
Low GI carbohydrate (Systain® 550)	1.20 g
Citric acid	0.20 g
Malic acid	0.10 g
Artificial sweeteners (aspartame and acesulfame)	0.04 g
Flavour compounds	0.12 g

Example 3. Protein drink.

A protein-rich drink was prepared by adding 9.05 grams per 100 ml of the powder comprising the following ingredients:

Pea protein isolate	5.88 g
Hydrolysed pea protein (Hyfoama™PW)	1.30 g
Low GI carbohydrate (Systain® 735	1.20 g
Artificial sweetener (acesulfame, aspartame)	0.05 g
Stabilizer	0.20 g
Flavour compounds	0.42 g

Example 4. Sheeted snack.

A protein-rich sheeted snack was prepared by adding to 60 ml of water 100 grams of a powder containing following ingredients:

Pea powder	20 g
Pea protein isolate	57 g
Pea hydrolysate Hyprol™ 7102 Dev	10 g
Bakasnack	5 g
Polyglycerolester	1 g
Dietary fiber	5 g
Salt	2 g

The ingredients were mixed into a homogeneous batter prior to being sheeted and deep-fried.

CLAIMS

1. Use of a protein hydrolysate originating from a non-mammalian source in the manufacture of a composition for use in a method of preventing and/or treating human obesity, said method comprising ingesting a composition containing, calculated on dry matter:

10-100 wt% protein hydrolysate;

0-90 wt.% intact protein;

0-50 wt.% carbohydrate; and

wherein hydrolysed protein and intact protein together are present in a concentration (w/w) that exceeds the carbohydrate concentration (w/w).

2. Use of a protein hydrolysate originating from a non-mammalian source in the manufacture of a composition for use in a method of preventing and/or treating lipid metabolism disorders in humans, said method comprising ingesting a composition as defined in claim 1.

3. Use of a composition as defined in claim 1 in a cosmetic method of stimulating and/or improving body weight reduction in order to improve the human body appearance, said method comprising ingesting the composition.

4. Use according to any one of the preceding claims, wherein the composition contains the protein hydrolysate and the optional carbohydrate in a weight ratio of more than 1:3.

5. Use according to claim 4, wherein the composition contains the protein hydrolysate and the optional carbohydrate in a weight ratio of more than 1:1.

6. Use according to any one of the preceding claims, wherein the composition contains 0-30% carbohydrate by weight of dry matter.

7. Use according to any one of the preceding claims, wherein the composition contains 20-80% intact protein by weight of dry matter.

8. Use according to any one of the preceding claims, wherein the composition contains between 0.5 and 5% rapidly and/or moderately absorbed carbohydrates by weight of dry matter and optionally contains slowly absorbed carbohydrates.

9. Use or composition according to claim 8, wherein the rapidly absorbed carbohydrates are selected from glucose, sucrose, maltose, and mixtures thereof, the moderately absorbed carbohydrates are selected from fructose, mannose, maltodextrin, white flour, wheat flour, and mixtures thereof, and the slowly absorbed carbohydrates are selected from raw corn starch, high amylose corn starch, modified starch, and mixtures thereof.

10. Use according to claim 8 or 9, wherein the composition contains slowly absorbed carbohydrates in a concentration (w/w) that exceeds the combined concentration (w/w) of rapidly and moderately absorbed carbohydrates by at least a factor 2.

11. Use according to any one of the preceding claims, wherein the composition is selected from the group consisting of beverages, snacks and soups.

12. Use according to any one of the preceding claims, wherein the method comprises ingesting the composition between 0.5 and 2 hours prior to the next meal.

13. Use according to any one of the preceding claims, wherein the method comprises ingesting the composition in an amount equivalent to at least 5 g, preferably at least 10 g of the combination of protein hydrolysate and intact protein per serving.

14. Use according to any one of the preceding claims, wherein the protein hydrolysate is a vegetable protein hydrolysate, preferably a hydrolysate of a vegetable protein selected from the group consisting of pea protein, rice protein, soy protein and combinations thereof.

15. A nutritional beverage containing:

0.5-10 wt.% hydrolysed protein from a non-mammalian source;

0-10 wt.% intact protein;

0.5-5 wt.% carbohydrates;

artificial sweetener;

flavouring; and

at least 80 wt.% water;

wherein hydrolysed protein and intact protein together are present in a concentration (w/w) that exceeds the carbohydrate concentration (w/w).

16. Nutritional beverage according to claim 15, wherein hydrolysed protein and intact protein together represent at least 5 wt.% of the beverage.

17. A savoury snack product containing:

3-50 wt.% hydrolysed protein from a non-mammalian source;

10-80 wt.% intact protein;

0-50 wt.% carbohydrate;

0.3-3 wt.% salt; and

0.1-20 wt.% water;

wherein hydrolysed protein and intact protein together are present in a concentration (w/w) that exceeds the carbohydrate concentration (w/w).

18. Snack product according to claim 17, wherein the product contains 1-20 wt.% dietary fibre.

19. A soup containing:

0.5-20 wt.% hydrolysed protein from a non-mammalian source;

0.5-29 wt.% intact protein;

0-20 wt.% carbohydrate;

0.1-2 wt.% salt; and

at least 70 wt.% water;

wherein hydrolysed protein and intact protein together are present in a concentration (w/w) that exceeds the carbohydrate concentration (w/w).

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, MEDLINE, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	page 6, line 18-21 page 42, line 23-26 page 43, line 1-11; claim 35	1-19
X	EP 0 425 423 A (HOEIE LARS HENRIK ;NYCOMED AS (NO)) 2 May 1991 (1991-05-02)	1-7,11, 13,14,17
Y	the whole document	1-19

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

International application No
PCT/NL 03/00641

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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(54) Title: USE OF A MILK PROTEIN HYDROLYSATE IN THE TREATMENT OF DIABETES

(57) Abstract: Use of a milk protein hydrolysate which is preferably a whey protein hydrolysate or caseinoglycomacropeptide (CGMP) in a bioavailable form in the manufacture of a composition for the treatment or prevention of diabetes or syndrome X and a method of treatment or prevention of diabetes or syndrome X are described. The present invention also relates to a method for assessing proglucagon gene expression and GLP-1 release by a cell line derived from an adenocarcinoma of human caecum.

Use of a Milk Protein Hydrolysate in the Treatment of Diabetes

FIELD OF THE INVENTION

The present invention relates to the use of milk protein hydrolysates in the manufacture of a medicament for the treatment or prevention of diabetes or syndrome X and a method of treatment of diabetes or syndrome X which comprises administering an effective amount of a composition comprising milk protein hydrolysates. The present invention also relates to the use of sweet whey or acid whey proteins or protein hydrolysate in the manufacture of a medicament for the treatment or prevention of diabetes or syndrome X and a method of treatment of diabetes or syndrome X which comprises administering an effective amount of a composition comprising sweet or acid whey proteins or protein hydrolysate. Furthermore, the present invention also relates to the use of CGMP in the manufacture of a medicament for the treatment or prevention of diabetes or syndrome X and a method of treatment of diabetes or syndrome X which comprises administering an effective amount of a composition comprising CGMP.

In addition, the present invention relates to the use of NCI-H716 cells, obtained from a cell line derived from a poorly differentiated adenocarcinoma of human caecum (de Bruine et al, Virchows Archiv B Cell Pathol 62:311-320,(1992)), as a model to measure proglucagon gene expression and GLP-1 secretion.

BACKGROUND OF THE INVENTION

B. Chabance et al. (Biochimie **80**, 155-165, 1998) have shown that after eating, many peptides derived from α-, β- or κ-caseins, including CGMP, can be detected in stomach and blood and this indicates that it can cross the intestinal barrier.

Diabetes mellitus is a metabolic disorder characterised by the failure of body tissues to store carbohydrates at the normal rate. Resistance to the action of insulin is the most important factor to type II diabetes. When this resistance exceeds the capacity of the beta cells to produce insulin, a person becomes diabetic. During the last 70 years people suffering from diabetes have been greatly aided by receiving controlled amounts of insulin.

Historically, insulin has been administered by injection to combat diabetes. Administering an injection requires expertise, and compared to oral administration, injecting a medicament is not as safe, convenient or acceptable to the patient. In the light of these concerns it is clear that there is a need for new nutritional or therapeutic products which may be administered orally.

Proglucagon, synthesised by L-cells found in the distal ileum and colon, is known to be post-translationally processed into peptides including glucagon-like peptide-1 (GLP-1), a potent insulin secretagogue. In addition to potentiating glucose-induced insulin secretion, GLP-1 is known to stimulate proinsulin gene expression and proinsulin biosynthesis.

Other actions of GLP-1 include inhibition of glucagon secretion and gastric motility. GLP-1 can bind in the brain, promoting satiety and suppressing food intake. Increasing insulin secretion is a key goal in the treatment of type II diabetes and stimulation of endogenous release of GLP-1 is a potential/prospective alternative to intravenous administration.

Improving glucose control in diabetes can provide the advantage of reducing the associated risks of hyperglycaemia, including blindness, limb amputations, kidney failure and cardiovascular disorders.

A number of *in vitro* cell models of animal origin have been developed to study the regulation of GLP-1 secretion including a foetal rat intestinal cell culture, a isolated canine L cell, a secretin tumour cell (STC-1) cell line, and the GLUTag enteroendocrine cell line. While these models have provided useful information regarding the factors which regulate GLP-1 secretion and proglucagon expression, they suffer from the problem that they do not necessarily represent the same regulators and mechanisms which are active and occur in human L cells.

The present invention addresses the problems set out above.

SUMMARY OF THE INVENTION

Remarkably, it has now been found that a milk protein hydrolysate can induce the release of GLP-1 and it can be used to improve glucose homeostasis *in vivo*.

In fact, in contrast to known studies, NCI-H716 cells have now been employed, obtained from a cell line derived from a poorly differentiated adenocarcinoma of human caecum (de Bruine et al, Virchows Archiv B Cell Pathol 62:311-320,(1992)). Surprisingly, the NCI-H716 cell line has now is found to be a good model for the first study of potential secretagogues, which regulate human GLP-1 secretion, *in vitro*. Up to date, NCI-H716 cell line was not known to be suitable for this purpose. Nor is it known from any human cell line to be capable of releasing GLP-1. So far, only cell lines derived from animals were available to serve as *in vitro* models to study proglucagon gene expression and GLP-1 secretion. This property of said cell line will enable much simplified research on GLP-1 release. Furthermore, the results obtained by the use of a human cell line to conduct studies on the production or function of GLP-1 will be much more relevant than result derived from other animal models. In short, the NCI-H716 cell-line line derived from a poorly differentiated adenocarcinoma of human caecum is likely to become a key tool for studying proglucagon gene expression and GLP-1 secretion in human. This cell line is deposited and available at the ATCC (American Type Culture Collection) under the ATCC Number CCL-250. The Depositor is A. F. Gazdar and the tissue of origin is the caecum, it is derived from a colorectal adenocarcinoma.

Consequently, in a first aspect the present invention provides use of a milk protein hydrolysate which is capable of inducing release of GLP-1 in the manufacture of a composition for the treatment or prevention of diabetes or syndrome X.

In a second aspect the invention provides a method of treatment or prevention of diabetes or syndrome X which comprises administering an effective amount of a milk protein hydrolysate which is capable of inducing release of GLP-1.

In a third aspect, the present invention provides a model for the study of proglucagon gene expression and GLP-1 production by humans comprising cells obtained from a cell line derived from an adenocarcinoma of human caecum.

In further aspect, the present invention provides a method for assessing proglucagon gene expression and GLP-1 release in humans comprising a cell line derived from an adenocarcinoma of human caecum.

In a last aspect, the present invention teaches the use of a cell line derived from an adenocarcinoma of human caecum to assess proglucagon gene expression and GLP-1 release in vitro.

An advantage of the present invention is that it provides a composition, which can be administered orally. This is both safer and more convenient for the patient than conventional treatment by injection.

Another advantage of the present invention is the reduced risk of hypoglycaemic reactions. Conventional injection of insulin carries with it the undesirable side effect of hypoglycaemic reactions. The use of oral hypoglycaemic agents to augment insulin secretion can also result in hypoglycaemic reactions. Once the plasma glucose levels reach fasting values, GLP-1 no longer stimulates insulin release. The advantage of enhancing insulin release via GLP-1 secretion is that the action of GLP-1 is glucose dependent and therefore eliminates the risk of hypoglycaemia, i.e. the release of insulin is very fine-tuned with respect to the blood glucose levels actually present.

Yet another advantage is that GLP-1 remains active in persons with diabetes whereas the other incretin hormone, glucose dependent insulinotropic peptide (GIP) loses effectiveness in diabetes.

Still another advantage of the present invention is that it provides metabolic benefits in addition to the augmentation of insulin release. Conventional treatment raises insulin levels, but the present invention in addition increases insulin mRNA, increases beta-cell sensitivity, and lowers glucagon levels.

Another advantage of the present invention is that it provides a composition, which can regulate appetite and reduce food intake. This action is beneficial in control of diabetes and syndrome X.
Still another advantage of the present invention is that for the first time a human cell line for the study of proglucagon gene expression and GLP-1 release is used.

Additional features and advantages of the present invention are described in, and will be apparent from, the description of the presently preferred embodiments, which are set out below with reference to the drawings in which:

DESCRIPTION OF THE DRAWINGS

Figure 1 shows secretion of GLP-1 by NCI-H716 cells in response to incubation for 2 h with different doses of CGMP-Ca form. Secretion into the medium was normalised to the total GLP-1 content (secretion + cells) of the culture well and is expressed as a percentage of the control value.

Figure 2 shows secretion of GLP-1 by NCI-H716 cells in response to incubation for 2 h with different doses of CGMP-Na form. Secretion into the medium was normalised to the total cell content of the culture well and is expressed as a percentage of the control value.

Figure 3 shows secretion of GLP-1 by NCI-H716 cells in response to incubation for 2 h with different fractions of CGMP. Secretion into the medium was normalised to the total cell content of the culture well and is expressed as a percentage of the control value. The composition of the different fractions was the following:

Fraction 1) Hydrolysed CGMP, pure peptide material, no phosphorus, no sialic acid.

Fraction 2) Hydrolysed CGMP, high sialic acid, high phosphorus content. Sample is in the Na-form.

Fraction 3) CGMP fraction enriched in CMPa and CMPb, the phosphorylated compounds of CGMP. Sample is in the Na-form.

Fraction 4) CGMP fraction enriched in sialic acid. Sample is in the Ca-form.

Figure 4 shows the amount of GLP-1 released in the medium after a 2h incubation period in the presence of 5 mg/ml of sweet whey, acid whey and meat protein hydrolysates. To exclude a possible effect due to the alpha-lactose content of the fractions of whey protein hydrolysates, an equivalent alpha-lactose dose as the one contained in the different wheys is separately illustrated.

In Figure 4, The GLP-1 secreted was measured differently than in Figures 1 to 3, i.e. with a kit that only measures the active form of GLP-1, i.e. GLP-1(7-37) or GLP-1(7-36 amide), but not the degraded GLP-1(9-36 amide) form like in figures 1 to 3 (see methods).

DETAILED DESCRIPTION OF THE INVENTION

Within the context of this specification the word "comprises" is taken to mean "includes, among other things". It is not intended to be construed as "consists of only".

Within the context of this specification the term "milk protein hydrolysate" is taken to mean milk proteins that have been subjected to any sort of hydrolysis. Thus, such "milk protein hydrolysate" may even include intact proteins that escaped hydrolysis and also any sort of fractions of proteins as obtained by the treatment of hydrolysis.

Within the context of this specification the terms "sweet whey" and "acid whey" are also considered to be possible milk protein hydrolysates, because they are the product of enzymatic or acid hydrolysis of milk proteins. Whey, however, as is well known in the art, can also comprise intact protein as well as different fractions of hydrolysed protein.

CGMP is used as an abbreviation for caseino-glycomacropeptide and CGMP-Ca and CGMP-Na are used as abbreviations for the calcium salt and sodium salt thereof. An alternative name for caseino-glycomacropeptide is k-caseinoglyco-peptide.

CGMP is a milk protein hydrolysate. It is a sialylated macropeptide, which is formed by the action of rennet or pepsin on kappa-casein from the milk of mammals.

Preferably, the milk protein hydrolysate which is capable of inducing release of GLP-1 comprises CGMP, a mimetic, homologue or fragment thereof which retains the activity of CGMP.

Preferably, an embodiment of the milk protein hydrolysate comprises the calcium or sodium salt of CGMP.

Preferably, the composition comprises a source of carbohydrate, a source of fat and a source of protein. More preferably, it comprises from about 15 to about 25% protein, from about 10 to about 30% fat, and from about 40 to about 60% carbohydrate. Preferably, at least a portion of the protein is provided as protein from sweet whey or acid whey. More preferably, at least a portion of the protein is provided as caseinoglycomacropeptide (CGMP).

Preferably, the milk protein hydrolysate, which is capable of inducing release of GLP-1, comprises proteins that are present in sweet whey or acid whey.

Preferably the composition is incorporated into a food formula.

Preferably, the composition comprises from about 1 to about 50 grams, preferably from 5 to about 25 grams and most preferably from 5 to about 10 grams protein hydrolysate from sweet whey or acid whey, or CGMP alone, or acid whey without CGMP, or a mixture thereof per 100 g of food formula.

Preferably the composition is administered to provide sufficient whey protein or, CGMP alone or whey protein without CGMP, to improve glucose metabolism in humans or companion animals by increasing plasma GLP-1 levels and controlling glycemic response. The exact amount could be determined without difficulty by administering whey protein or, as an example, CGMP until the correct effect is seen. The dose of whey protein comprising or not comprising CGMP or of CGMP itself is preferably from about 1 to about 50 grams per day,

more preferably from 9 to about 18 grams per day and most preferably from 3 to about 6 grams consumed at three times throughout a day.

It is well known in the art how a milk or whey protein can be obtained. In general, skimmed milk is treated with enzymes or acid in order to finally separate sweet or acid whey, respectively, which is thus deprived from the clotted casein. The sweet or acid whey then comprises whey protein hydrolysates and also minor proteins, which remain intact. Thus, sweet or acid whey is, for example, obtained as a side product from production of cheese. Although it is not necessary to additionally process whey to work the present invention, it is obvious to the skilled person that further processing is possible. For example, sweet and acid fluid whey can be condensed, dried, fermented, delactosed, demineralized and deproteinated. In order to work the present invention it is, for example, possible to use whey concentrate or whey powder. The latter is especially convenient to be added to any chosen food product to cause the desired effect. It is also clear to the skilled person, that protein hydrolysate present in sweet or acid whey can be further hydrolysed, for example to prepare a hypoallergenic whey protein hydrolysate. According to U.S. Pat. No. 5,039,532, whey protein material is subjected to a second hydrolysis with a proteolytic enzyme in order to hydrolyse the minor proteins remaining intact after the first hydrolysis. Such a hydrolysate may then be used as a liquid or it may be dried and incorporated in numerous food products.

Preferably CGMP is obtained by an ion-exchange treatment of a liquid lactic raw material containing CGMP. Suitable starting materials of lactic origin may include for example:

- the product of the hydrolysis with rennet of a native casein obtained by acidic precipitation of skimmed milk with a mineral acid or acidifying ferments, optionally with addition of calcium ions,
- the hydrolysis product of a caseinate with rennet,
- a sweet whey obtained after separation of casein coagulated with rennet,
- a sweet whey or such a whey demineralised, for example, by electrodialysis and/or ion exchange and/or reverse osmosis,
- a concentrate of sweet whey,
- a concentrate of whey proteins obtained by ultrafiltration and diafiltration of sweet whey.

- mother liquors of the crystallisation of lactose from a sweet whey,
- a permeate of ultrafiltration of a sweet whey.

A preferable method of obtaining CGMP is described, for example, in WO 98/53702 and includes the decationization of the liquid raw material, such that the pH has a value of 1 to 4.5, bringing the said liquid into contact with a weak anionic resin of hydrophobic matrix, predominantly in alkaline form up to a stabilised pH, then separation of the resin and the liquid product which is recovered, and desorption of CGMP from the resin.

Preferably, an embodiment of the composition comprises a milk protein hydrolysate. It has been shown that skimmed milk results in CGP levels of 1.1μg/ml in human plasma. After yoghurt ingestion 2.8μg/ml of CGP has been detected in blood.

Preferably, an embodiment of the composition comprises CGMP.

Preferably, an embodiment of the composition comprises sweet or acid whey, more preferably, intact or partially hydrolysed proteins from sweet or acid whey.

Preferably an embodiment of the composition comprises a source of protein and at least protein hydrolysates from sweet whey or acid whey, or CGMP. Dietary protein is preferably used as a source of protein. The dietary proteins may be any suitable dietary protein; for example animal protein (such as milk protein, meat protein or egg protein); vegetable protein (such as soy protein, wheat protein, rice protein, or pea protein); a mixture of free amino acids; or a combination thereof. Milk protein such as casein, whey protein or soy protein is particularly preferred.

The composition may also contain a source of carbohydrate and/or a source of fat.

A preferred embodiment of the composition comprises a fat source, the fat source preferably provides about 5% to about 55% of the energy of the nutritional formula; for example about 20% to about 50% of the energy. The lipids making up the fat source may be any suitable fat or fat mixture. Vegetable fat is particularly suitable; for example soy oil, palm oil, coconut oil, safflower oil,

sunflower oil, corn oil, canola oil, lecithins, or the like or a mixture thereof. Animal fat such as milk fat may also be added if desired.

A preferred embodiment of the composition comprises a source of carbohydrate. It preferably provides about 40% to about 80% of the energy of the nutritional composition. Any suitable carbohydrate may be used, for example sucrose, lactose, glucose, fructose, corn syrup solids, and maltodextrins, or a mixture thereof.

A preferred embodiment of the composition comprises dietary fibre. If used, it preferably comprises up to about 5% of the energy of the nutritional formula. The dietary fibre may be from any suitable origin, including for example soy, pea, oat, pectin, guar gum, gum arabic, or fructooligosaccharide.

A preferred embodiment of the composition comprises one or more suitable vitamins and/or minerals may be included in an embodiment of the composition in an amount to meet the appropriate guidelines.

A preferred embodiment of the composition comprises one or more food grade emulsifiers may be incorporated into the nutritional formula if desired; for example diacetyl tartaric acid esters of mono- and di- glycerides, lecithin and mono- and di-glycerides. Similarly suitable salts and stabilisers may be included.

A preferred embodiment of the composition is enterally administrable; for example in the form of a powder, a liquid concentrate, or a ready-to-drink beverage. If it is desired to produce a powdered nutritional formula, the homogenised mixture is transferred to a suitable drying apparatus such as a spray drier or freeze drier and converted to powder.

In a further embodiment, a typical food product may be enriched with whey protein or CGMP. For example, a fermented milk, a yoghurt, a fresh cheese, a renneted milk, a confectionery bar, breakfast cereal flakes or bars, drinks, milk powders, soy-based products, non-milk fermented products or nutritional supplements for clinical nutrition. Then, the amount of whey protein or of CGMP added is preferably of at least about 0.01% by weight.

In an alternative embodiment the composition may be incorporated in an article of confectionery, for example a sweet, or sweetened beverage.

MATERIALS AND METHODS

Materials for the cell culture:

RPMI 1640 medium, Dulbecco's Modified Eagles medium (DMEM), additives and foetal bovine serum (FBS) were from Gibco (Life Technologies, Basel, Switzerland). Bovine serum albumin (BSA) was purchased from Serological Proteins Inc. (Kankakee, IL).

Materials for testing the effect of CGMP:

CGMP was obtained from R&D Konolfingen and was dissolved directly in Krebs Ringer Buffer. Two forms of CGMP, sodium extracted and calcium extracted, were tested as well as four fractions of CGMP.

Materials for testing the effect of sweet and acid whey:

For this study, conventional milk fractions at lab scale from fresh bovine milk (local market) were prepared.
Rennet (Presure simple) was from Rhône Poulenc Rorer (Cooperation Pharmaceutique Française, 77000 Melun France, Batch N°101089007 expire date 2000.09.21) 50 mg active chymosine by liter. Produced by TEXEL 38470 Vinay France. Furthermore, CaCl2 2H2O, HCl 32%, Acetic acid (glacial), Sodium Hydroxide were used.

Milk fractions

Bovine milk fractions

Lab scale fractionments were adapted from conventional milk processes. Centrifugation was realised at higher acceleration rate and non-soluble fractions were washed to increase selectivity and fractionment efficacy.

Cream; cream washing and skimmed milk

Usually cream was extracted from whole milk by centrifugation between 3 000 and 4 500 g, the selectivity of this step was improved by increasing acceleration up to 13 600 g using fixed angle rotor Sorval GS3 at 9 000 rpm during 30 min. Starting from 2 200 ml of whole milk 90g of cream were recovered on the top layer.

Cream washing (3 times labelled respectively, *cream washing1, 2 and 3*): the cream layer was dispersed in 3 water volumes (270 ml) and gently scattered in bottle by manual shaking before subsequent centrifugation.

Butter particles were spontaneously formed at the top of the bottle after the third cream washing and the buttermilk was recovered by sticking butter particles together.

A non-soluble fraction was recovered after centrifugation on the bottom of bottles used for cream washings (Labelled: *washed cream sediments*)

Sweet whey, rennet casein washing and rennet casein

The separation whey/casein is obtained by enzymatic treatment of skimmed milk inducing casein clotting. 520µl of CaCl2 200mM were added to 520g of skimmed milk to reach 2 mM final concentration added. This skimmed milk was heated at 35 °C then 250µl of rennet were immediately added under moderate magnetic stirring. After 1 min the blend was incubated 50 min at 35°C in water bath, poured in bottles for subsequent centrifugation (13 600g 30 min) to separate sweet whey from the non-soluble rennet casein.

The 476 g of supernatant were fractionated in 10 X 1.3 ml aliquots (ependorf) and 40 ml plastic tubes. Labelled (*Sweet whey*) and freezed by immersion in liquid nitrogen and stocked in plastic bag at minus 20°C.

The rennet casein (45g) was dispersed in 286 ml CaCl2 2mM NaCl 0.9%, centrifuged, the supernatant (246 ml) was aliquoted, labelled (*rennet casein washing*) and freezed in liquid nitrogen.

The 31g recovered rennet casein were dispersed in CaCl2 2mM NaCl 0.9%, volume was adjusted to 250 ml, aliquoted, labelled (*rennet casein*) and freezed.

Acid whey, acid casein washing and acid casein

The separation whey/casein is obtained by chemical acidification of skimmed milk inducing casein clotting. 520µl of CaCl2 200mM were added to 520g of skimmed milk to reach 2 mM final concentration added. This skimmed milk was acidified at 25°C by addition of 32% HCl from pH 6.6 to pH 4.6 °C under moderate magnetic stirring. After 1 min of stirring the blend was incubated 60 min at 25°C, poured in bottles for subsequent centrifugation (13 600g 30 min) to separate acid whey from the non soluble acid casein.
The 503 g of supernatant were fractionated in 10 X 1.3 ml aliquots (ependorf) and 40 ml plastic tubes. Labelled (*Acid whey*) and freezed by immersion in liquid nitrogen and stocked in plastic bag at minus 20°C.
The acid casein (41g) was dispersed in 233 ml 20mM Sodium acetate pH 4.6, centrifuged, the supernatant (250 ml) was aliquoted, labelled (*Acid casein washing*) and freezed in liquid nitrogen.
The 28.6g recovered acid casein were dispersed in water, pH adjusted from 4.67 to 6.6 by NaOH addition and volume was adjusted to 250 ml, aliquoted, labelled (*Acid casein*) and freezed.

Cell line and culture conditions:

The human NCI-H716 cells, originally developed from a poorly differentiated caecal adenocarcinoma, were obtained from the American Type Culture Collection (ATCC, Rockville, Maryland, USA). Cells were grown in suspension at 37°C, 5% CO_2. The culture medium consisted of RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin. Endocrine differentiation is enhanced *in vitro* in NCI-H716 cells grown on an extracellular matrix (de Bruine et al, 1993). Cells were, therefore, seeded in dishes coated with Matrigel® (Becton Dickinson, Bedford, MA, USA) two days before experiments.

Secretion Studies:

Two days before experiments, $1x10^6$ cells were seeded in 12 well culture plates coated with Matrigel®. On the day of the experiment, the supernatant was replaced by Krebs-Ringer Bicarbonate Buffer (KRB) containing 0.2% wt/vol

BSA with or without CGMP. Cells were incubated for 2 h at 37°C. Supernatants were collected with the addition of 50 µg/ml PMSF and frozen at –80°C for subsequent analysis by radioimmunoassay (RIA) of GLP-1. Cells were scraped with a rubber policeman and homogenisation buffer [1 N HCl containing 5% (v/v) HCOOH, 1% (v/v) trifluoroacetic acid (TFA), and 1% (v/v) NaCl] and sonicated for 15s. Peptides were extracted from the cell medium and cell homogenates using an alcohol extraction as described by the supplier of the GLP-1(7-36) Total RIA Kit (Linco Research Inc., St. Charles, MO, USA). Concentrations of GLP-1 (Total, i.e., 7-36 amide or 9-36 amide) were measured using a commercial RIA kit (Linco Research Inc., St. Charles, MO, USA). This kit measures GLP-1(7-36)NH_2 and GLP-1(9-36)NH2 with less than 0.4% crossreactivity with GLP-1(7-37). The ED_{50} for the assay was 72 pM. The intraassay coefficient of variance was 2.28%.

For testing the effect of whey and meat hydrolysates, (figure 4), the GLP-1 secreted was measured differently than before, i.e. with a commercial ELISA kit (Linco Research Inc., St. Charles, MO, USA). This kit measures the active form of GLP-1, i.e. GLP-1(7-37) or GLP-1(7-36 amide), but not the degraded GLP-1(9-36 amide) form like in figures 1 to 3.

RESULTS

CGMP stimulates the release of GLP-1 in the NCI-H716 intestinal cell line.

The amount of GLP-1 released into the medium of NCI-H716 cells treated for 2h with increasing concentrations (0.25-2.5 mg/ml wt/vol) of the calcium form of CGMP is shown in Fig. 1. CGMP induced a dose-dependent increase in GLP-1 concentrations with maximum secretion reaching 259 ± 77% (n = minimum of 3) of the control values with 2.5 mg/ml of CGMP-Ca. The symbol * represents a significant difference from control values ($p<0.05$).

Figure 2 shows the amount of GLP-1 released into the medium of NCI-H716 cells treated for 2h with increasing concentrations (0.25-2.5 mg/ml wt/vol) of the sodium form of CGMP. CGMP induced an increase in GLP-1 concentrations with maximum secretion reaching 255 ± 41% (n = minimum of 3) of the control

values with 2.5 mg/ml of CGMP-Ca. The symbol * represents a significant difference from control values ($p<0.05$).

Figure 3 shows the amount of GLP-1 released into the medium of NCI-H716 cells treated for 2h with 1 mg/ml (wt/vol) of different fractions of CGMP. All fractions, except fraction 3 ($p=0.085$) significantly increased GLP-1 secretion with Fraction 2 resulting in the highest stimulation of 220 ± 41% (n=3) of the control values. The symbol * represents a significant difference from control values ($p<0.05$).

Sweet and acid whey stimulate the release of active GLP-1 in the NCI-H716 intestinal cell line.

The amount of GLP-1 released in the medium after a 2h incubation period in the presence of 5 mg/ml milk protein hydrolysates is shown in figure 4. Sweet whey and acid whey induced an increase in GLP-1 release of 298 ± 34 % and a 284 ± 21 %, respectively, compared to control condition ($p = 0.03$ and 0.01 respectively, $n = 3$). This effect was not due to the alpha-lactose content of these fractions, as an equivalent alpha-lactose dose as the one contained in the different wheys only resulted in a small raise in GLP-1 secretion (144 ± 32 %, compared to control). Moreover, another protein hydrolysate, meat hydrolysate, didn't induce such an effect on GLP-1 production, at 5 mg/ml (132 ± 6 %, compared to control).

The following examples are given by way of illustration only and in no way should be construed as limiting the subject matter of the present application. Percentages and parts are by weight unless otherwise indicated.

Example 1: Preparation of CGMP.

Bovine sweet whey was concentrated to 17% dry matter, demineralised by electrodialysis, freed of cations on a strong cationic resin column, freed of anions on a weak anionic resin column and spray-dried in a drying tower. Its composition is indicated below:

	%
Proteins (GMP included)	11.7
Lactose	81.7
Ash	1
Lipids	1
Water	balance for 100

The demineralized whey powder was solubilized in deionized water. After cation removal the solution has an initial pH of 3.8. In the preceding plant, 392 kg of this solution was treated at the temperature of 8°C, while stirring it in the reactor in the presence of 23 kg of weak anionic resin of hydrophobic matrix based on polystyrene (IMAC HP 661®, Rohm & Haas, regenerated in OH- form) for 4 h. Stabilization of the pH at 4.89 indicates the end of the reaction. The liquid was drawn off and the resin was recovered as above.

After concentration of the liquid to 45% dry matter by evaporation, the concentrate was spray-dried in a drying tower.

Analysis of the concentrate by HPLC showed that the reaction removed 89% of the starting CGMP. Moreover, the powder contained 9.1% of whey protein, which corresponded to a yield of 90% of the whey proteins.

To recover CGMP, the resin was washed successively with deionized water, with 30 l of an aqueous solution at 0.5% HCl and with 30 l of deionized water, and the CGMP was eluted twice with 40 l of aqueous solution at 2% Ca(OH)2. Rinsing is carried out with 30 l of deionized water. After combining the eluate and rinsing volumes, the combination was concentrated to a volume of 25 l by ultrafiltration with a membrane having a nominal cut-off of 3000 daltons. The retentate was freeze-dried and 900 g of CGMP were obtained, corresponding to a yield of 80% relative to the starting CGMP.

Example 2 : Fermented milk containing CGMP or whey powder

A traditional fermented milk with 1-4 % fats was prepared as follows:
After standardising whole milk, low fat milk or a mixture of both, 0.05% by weight of CGMP as prepared in example 1 are added. The whole was pasteurised

in a plate exchanger, the liquid was cooled to the fermentation temperature, a thermophilic or mesophilic lactic ferment was added and incubation was carried out until a pH of <5 was obtained.

Subsequent filling and sealing pots took place in a conventional manner.

Alternative embodiments having additions of 0.1 %, 0.25 % and 0.5% by weight of CGMPs and commercial whey powder have been prepared.

Example 3 : Fermented and gelled milk enriched in probiotic bacteria containing CGMP or whey powder

Fermented and gelled milks were prepared enriched in probiotic bacteria. 89.3 parts milk containing fat were mixed with 3.7 parts of skimmed milk powder and about 0.05 by weight of CGMP as prepared in example 1, then the mixture was preheated to 70°C and pasteurised at 92°C/6 min, and after having been cooled to 43°C the mixture was inoculated with 2% of a common yoghurt starter comprising Streptococcus thermophilus and Lactobacillus bulgaricus and with 5% of Lactobacillus johnsonii (La-1, CNCM I-1225). After conditioning in pots, fermentation was carried out at 38°C up to pH 4.6 and the pots were then cooled to 6°C.

The following amounts of CGMP or commercial whey powder were added: 0.1 %, 0.25 % and 0.5% by weight.

Example 4 : Fermented and gelled milk enriched in probiotic bacteria containing CGMP or whey powder

Fermented and gelled milks are prepared as described in the previous example, wherein Lactobacillus johnsonii strain is replaced by Lactobacillus acidophilus La-10 (Nestlé Culture collection, Lausanne, Switzerland) (ATCC 11975).

Example 5 : Enteral composition containing CGMP

An enteral composition with an energy density of 6.3 kJ/ml and 8% (p/v) of proteins was prepared from "low temperature" skimmed milk powder, i.e. skimmed milk dried under controlled thermal conditions.

20 kg of low temperature skimmed milk powder was dispersed in 100 kg of demineralised water at a temperature of about 50-55°C. This dispersion is microfiltered by passing demineralised water through until 600 kg of permeate have been eliminated. The retentate is then further concentrated to around 60 kg, which represents a dry matter content of 21% with a protein content, based on dry matter, of 82%.

To prepare the enteral composition, 2.3 kg of liquid retentate are mixed at 55°C with 600 g of maltodextrin, 200 g of sucrose, 20.3 g of Tri-K citrate H_2O, 9.2 g of $MgCl_26H_2O$, 5.8 g of NaCl and about 0.5 to 1 % by weight of CGMP as prepared in example 1 or, instead of CGMP, commercial whey powder.

After the ingredients were dissolved in the retentate, demineralised water is added to a total weight of the dispersion of 4.7 kg. The pH was adjusted to 6.8, after which 300 g of fatty phase are introduced, the total weight of the dispersion being 5 kg.

After homogenisation and sterilisation, the product had an agreeable sugary taste.

Example 6 : Cereal bar containing CGMP or whey powder

In order to prepare an expanded starting product, barley, wheat, corn or oat flour was treated in a twin-screw extruder for about 15 seconds at a screw speed of about 350 r.p.m. in the presence of approximately 12% of water. After the treatment, the expanded product left the extruder in the form of 2 to 3 mm long granules which were dried for 20 minutes at 100°C. The product thus obtained had a cellular structure and has the following composition:

Edible fibers	31%
Proteins	21%
Glucides	37.5%
Lipids	6.5%
Ash	2.4%
Water	1.6%

The expanded product was incorporated in a bar intended for treatment of diabetes, which had the following composition:

Expanded product	39.4%
Oat flakes	16.7%
Sorbitol	8.4%
Fructose	8.5%
Apple cubes	6.1%
Rice crispies	4.1%
Gelatine	4.0%
Apricot powder	2.5%
Palm oil	3.0%
CGMP as prepared in example 1	2.5%
Water	4.8%

Example 7: Food supplement containing CGMP

A culture of the strain Lactobacillus johnsonii La-1 (CNCM I-1225) of human origin, was mixed with CGMP as prepared in example 1 and spray dried according to the process given in EP0818529 so as to obtain a food supplement containing an amount of about 5% by weight of CGMP.

The powder obtained may be used as a food supplement. A breakfast cereal, milk product or another food product may then be sprinkled with this powder containing CGMP.

Example 8: Food supplement containing CGMP

A food supplement was prepared as described in example 9. However, Lactobacillus johnsonii was replaced by Lactobacillus acidophilus, La-10 (Nestec collection, Lausanne, Switzerland) or a mixture of the two strains.

It should be understood that various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present invention and without diminishing its attendant advantages. It is therefore intended that such changes and modifications be covered by the appended claims.

CLAIMS

1. Use of a milk protein hydrolysate or compound including a milk protein hydrolysate, which is capable of inducing release of GLP-1, in a bioavailable form in the manufacture of a composition for the treatment or prevention of diabetes or syndrome X.

2. Use according to claim 1 wherein the milk protein hydrolysate is caseinoglycomacropeptide (CGMP), a mimetic, homologue or fragment thereof.

3. Use according to claim 2 wherein CGMP is in the form of a calcium or sodium salt.

4. Use according to any preceding claim wherein the composition comprises an amount of about 0.01% to about 10% by weight dry matter of CGMP.

5. Use according to claim 1 wherein the milk protein hydrolysate is sweet whey or acid whey.

6. Use according to claim 5 wherein the composition comprises an amount of 0.01% to about 10% by weight dry matter of sweet or acid whey.

7. A method of treatment or prevention of diabetes or syndrome X which comprises administering an effective amount of a milk protein hydrolysate which is capable of inducing release of GLP-1.

8. A method according to claim 7 wherein the milk protein hydrolysate which is capable of inducing release of GLP-1 is CGMP, a mimetic, homologue or fragment thereof

9. A method according to claim 8 wherein the CGMP is in the form of a calcium or sodium salt.

10. A method according to claim 7, wherein an effective amount of sweet or acid whey, or further processed sweet or acid whey is administered.

11. A model for the study of proglucagon gene expression and GLP-1 production by humans comprising cells obtained from a cell line derived from an adenocarcinoma of human caecum.

12. A model according to claim 11, characterised in that said cell line is the NCI-H716 cell line, having the ATCC number CCL-251.

13. A method for assessing proglucagon gene expression and GLP-1 release in humans comprising a cell line derived from an adenocarcinoma of human caecum.

14. A method according to claim 13, characterised in that said cell line is the NCI-H716 cell line, having the ATCC number CCL-251.

15. The use of a cell line derived from an adenocarcinoma of human caecum to assess proglucagon gene expression and GLP-1 release in vitro.

16. The use according to claim 15, characterised in that said cell line is the NCI-H716 cell line, having the ATCC number CCL-251.

Figure 1

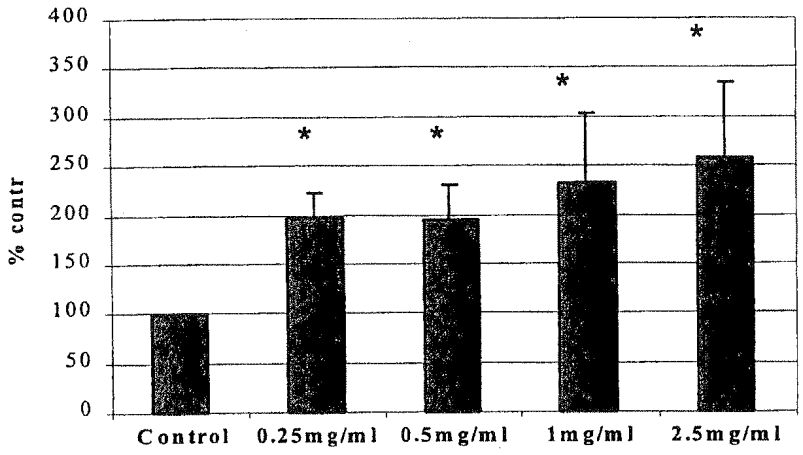
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Figure 2

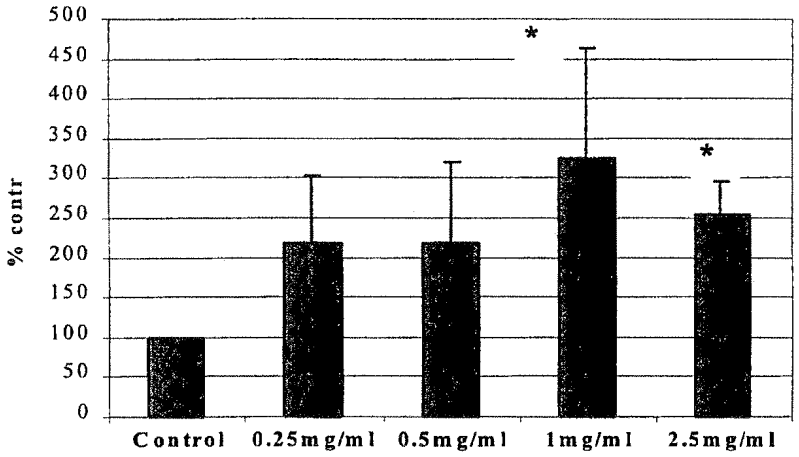
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Figure 3

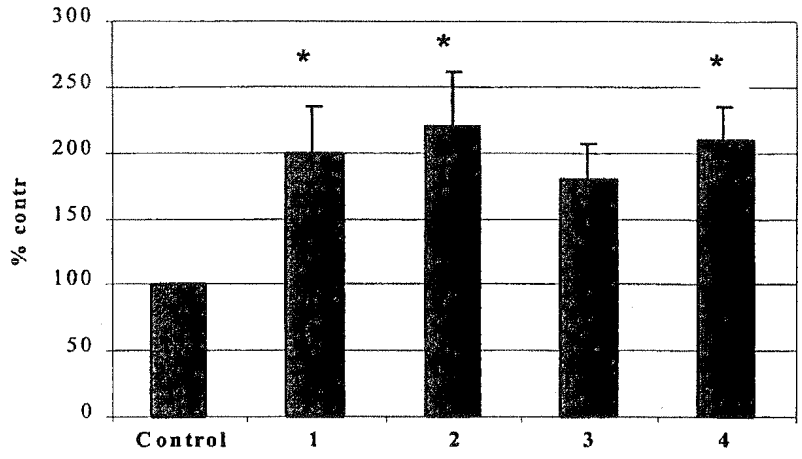
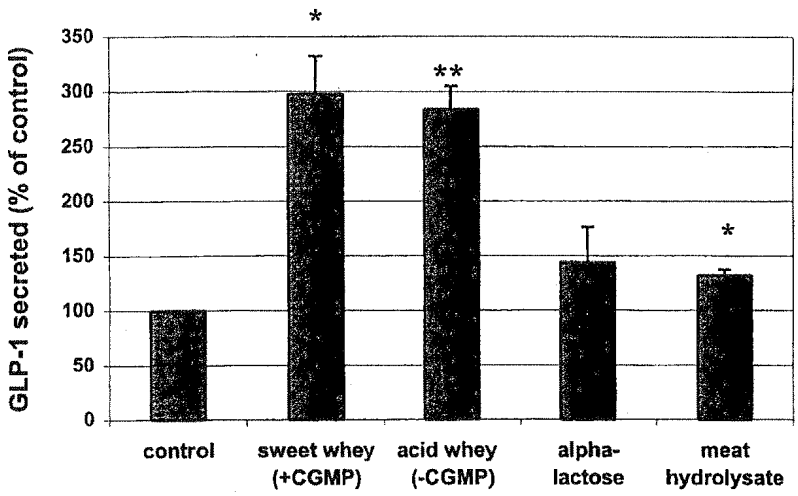


Figure 4


GLP-1 secreted (% of control)
350
300
250
200
150
100
50
0
*
**
*
control
sweet whey (+CGMP)
acid whey (-CGMP)
alpha-lactose
meat hydrolysate

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— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

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(54) Title: COMPOSITION FOR TREATING AND/OR PREVENTING DYSFUNCTIONS ASSOCIATED WITH TYPE 2 DIABETES MELLITUS

(57) Abstract: The present invention relates to the use of a composition for treating, preventing and/or improving metabolic dysfunctions associated with Type 2 diabetes mellitus and insulin resistance, said composition comprising intact whey protein, and to nutritional or pharmaceutical compositions and functional food products.

WO 2005/000325 A2

Composition for treating and/or preventing dysfunctions associated with Type 2 diabetes mellitus

The present invention relates to the treatment and/or prevention of dysfunctions associated with Type 2 diabetes mellitus and/or insulin resistance.

Diabetes mellitus and insulin resistance both are metabolic disorders exhibiting a major common manifestation, hyperglycaemia.

Diabetes mellitus originates from an inherited and/or acquired deficiency in the production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency eventually results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves.

There are two principle forms of diabetes, Type 1 and Type 2.

In Type 1 diabetes the pancreas of affected individuals fails to produce insulin largely due to a destruction of the islets of Langerhans, which in most cases seem to occur as a consequence of an auto-immune reaction triggered by some environmental factor, such as a viral infection. Heavy lymphocytic infiltrates appear in and around islets with the number and size of islets being reduced, eventually leading to decreased insulin production and glucose intolerance. This form develops most frequently in children and adolescents, but is being increasingly noted later in life.

Type 2 diabetes results from the body's inability to properly respond to the action of insulin produced by the pancreas. It occurs most frequently in adults, but is being noted increasingly in adolescents as well. The islets of Langerhans are normal in number or somewhat reduced with type II diabetes mellitus. Fibrosis and deposition of amylin polypeptide within islets are most characteristic of the chronic states of Type 2 diabetes.

Diabetes mellitus of both types is associated with a number of life-threatening and/or handicapping diseases. Examples are nodular and diffuse glomerulosclerosis, which may lead to chronic renal failure. Diabetics are prone to infections, particularly pyelonephritis. Also the eyes may be affected with diabetic retinopathy being one of the leading causes for irreversible blindness. Most persons with Type 1 diabetes and many of those with Type 2 diabetes develop some sort of background (non-proliferative) retinopathy. In severe cases, neo-vascularization may lead to adhesions (synechiae) between iris and cornea or iris and lens, eventually leading to secondary glaucoma with blindness. Also cataracts are more common in diabetics. This predilection for development of cataracts is felt to result from hyperglycaemia leading to accumulation of sorbitol that results in osmotic damage to the crystalline lens.

Persons with diabetes mellitus, either Type 1 or Type 2, also exhibit early and accelerated atherosclerosis. The most serious complications of this are atherosclerotic heart disease, cerebrovascular disease, and renal disease, with the most common cause of death being myocardial infarction. Peripheral vascular disease is a particular problem with diabetes mellitus and is made worse through the development of diabetic neuropathy, leading to propensity for injury. Mucormycosis is another feared complication in individuals experiencing diabetes mellitus. The site of involvement is typically the nasopharyngeal region, but the infection can spread to involve soft tissues and bone of the face, orbit, skull, and brain.

The treatment of individuals suffering from diabetes generally involves physical activity, diet and/or administration of medicaments. People with Type 1 diabetes are usually totally dependent on insulin injections for survival, requiring daily administration. Type 2 diabetic patients usually have to observe a strict diet and may additionally receive oral anti-diabetics, such as sulphonyl ureas, alpha-glucosidase inhibitors and biguanides, or even injections of insulin, the administration of which is often associated with severe side effects and complications.

The majority of people suffer from Type 2 diabetes, which accounts for around 90% of all diabetes cases world-wide. On the molecular level Type 2 diabetes is characterized by a defect of both, insulin secretion and action. The defect of insulin secretion relates mostly to the first phase of the post-prandial insulin release from pancreas, wherein in diabetic patients the already formed insulin is stored within the β-cells, but cannot be released into circulation. Indeed, most of the Type 2 diabetic patients present a resistance to the action of the insulin such that in order to cope with similar glucose concentration as present in healthy people, Type 2 diabetics require a higher concentration of insulin in plasma.

Another type of abnormalities in glucose metabolism is insulin resistance, that is, a reduced sensitivity in the tissues of the body to the action of insulin, which goes along with a perturbed lipid (blood fats) metabolism, obesity, and high blood pressure. This cluster of abnormalities has come to be known as a syndrome, going by a variety of names, including Syndrome X, the Deadly Quartet, and the Insulin Resistance Syndrome.

When insulin resistance, or reduced insulin sensitivity, exists, the body attempts to overcome this resistance by secreting more insulin from the pancreas. The development of Type 2, or non-insulin dependent, diabetes occurs when the pancreas fails to sustain this increased insulin secretion. The importance of the Insulin Resistance Syndrome, or perhaps more accurately, "The Pluri-Metabolic Syndrome", lies in its consequences. The syndrome is typically characterized by varying degrees of glucose intolerance, abnormal cholesterol and/or triglyceride levels, high blood pressure, and upper body obesity, all independent risk factors for cardiac disease.

Following a meal, a person suffering insulin resistance will have elevated glucose circulating in the blood, signalling yet more insulin to be released from the pancreas until the glucose is taken up by the cells. Experts suggest that 11 to 25 percent of the adult population may be resistant to insulin to some degree.

The use of milk protein hydrolysates to improve glucose metabolism or control glycaemic response in individuals suffering from diabetes has already been suggested in US Patent Application No. 2003/0004095. According to this document, milk protein hydrolysates, specifically caseinoglycomacropeptide can induce the release of GLP-1, a peptide known to be capable of potentiating glucose-induced insulin secretion as well as stimulating proinsulin gene expression and proinsulin biosynthesis. However, milk protein hydrolysates are known to have an unpleasant bitter taste which may adversely affect patient compliance with a regime based on these products.

Due to the increasing number of affected people world-wide and the changing lifestyle of the society there exists a need in the art to provide additional means useful in preventing, treating and/or improving conditions associated with Type 2 diabetes mellitus and/or insulin resistance. Moreover, such a means should be essentially free from disadvantageous side-effects well known from many oral anti-diabetics, and should be easy to take up.

The present invention provides a method of treating, preventing and/or improving metabolic dysfunctions and conditions associated with Type 2 diabetes mellitus by administering an effective amount of a composition containing intact whey proteins

The inventors have found that intact whey proteins induce a dramatic but short increase in plasma amino acids as opposed to proteins such as casein which are more slowly digested and which induce a mild but prolonged plateau of hyperaminoacidemia (Boirie et al, Proc. Natl. Acad. Sci. USA, 1997, 94:14930-5).

Intact whey protein may be present in the composition in an amount of from about 1 to 90 % by weight, preferably from 5 to 70 % by weight, more preferably 11 to 60 % by weight, even more preferably 21 to 40 % by weight and most preferably about 25 to 35 % by weight, on the basis of the total dry weight of the composition. Preferable the whey protein is sweet whey protein.

It has been found that intact whey proteins have a particular effect after consumption by type 2 diabetes patients. According to the studies carried out intact whey proteins significantly increase the production and/or secretion of insulin, as determined by an increase in the maximal plasma concentration and bio-availability of pro-insulin, insulin and C-peptide. The C-peptide results from the formation of biological active insulin from pro-insulin and serves as an indicator showing how much insulin is produced in an individual. C-peptide is considered to represent the most accurate indicator for the production of insulin in ß-cells. In other words, intact whey proteins enhance post-prandial insulinemia and help to restore the first phase of the insulin response of diabetic patients to a standard meal and the kinetics of post-prandial insulinemia provoked by dietary carbohydrates may thus be accurately modulated by such proteins.

The present composition will also be of high interest for large parts of the population, which are not suffering from insulin resistance or Type 2 diabetes mellitus at present, but belong to a target group at risk to develop any of said disorders, either due to a high risk diet or genetic predisposition. Moreover, an enhancement of post-prandial insulinemia is also highly interesting for other groups of persons, such as patients recovering from diseases or trauma leading to muscle depletion, exercising persons or elderly persons, since insulin is an anabolic hormone necessary for muscle mass maintenance and growth. High post-prandial insulinemia therefore promotes improving muscle mass accretion in exercising persons, is helpful for patients suffering from muscle depletion, and supports muscle maintenance in elderly persons.

The composition as described above may of course also be used for the manufacture of a so called functional food product or a pharmaceutical composition.

Particularly good results may be achieved when providing at least 0.1 g intact whey proteins per kg body weight, more preferably 0.5 to 0.8 g intact whey proteins per kg body weight, e.g. during, before or after a standard meal, in particular a standard meal

comprising carbohydrates. A standard meal is any meal comprising at least 150 kcal, more preferably at least 250 kcal.

The composition is preferably enterally administrable, such as in form of a powder, a liquid concentrate, or a ready-to-drink beverage. The composition can be directly consumed or admixed with various foodstuffs, in particular to ready-to-use snacks, dairy products or drinks, or used for the preparation of an oral or enteral nutritional composition or a fruit juice.

The composition according to the present invention may of course comprise other conventional ingredients, such as vitamins and minerals, dietary fibres, fat, food additives etc..

In particular, vitamins and minerals may be present in an amount of between 30% and 150% of US RDA (US recommended (daily) dietary allowance) per daily dosage. Additionally, one or more food grade emulsifiers may be included in the nutritional composition, if desired, such as diacetyl tartaric acid esters of mono- and diglycerides, lecithin, and mono- or diglycerides or a mixture thereof. Similarly, suitable food-acceptable salts and/or stabilizers may also be included.

If desired, fibres either soluble and insoluble may be included.

If a lipid source is included, it preferably comprises about 5% to 40 % of the energy (measured in calories) on the basis of the total energy of the composition; preferably, about 10 % to about 20 % of the energy. Any suitable fat or fat mixture may be used. Vegetable fat is particularly suitable, for example soy oil, palm oil, coconut oil, safflower oil, sunflower oil, corn oil, canola oil, lecithin and the like. Animal fat such as milk fat may also be added if desired.

If a carbohydrate source is included, it preferably comprises less than 10% by weight, preferably less than 5% by weight, more preferably less than 1% by weight of the

composition. For some applications, such as e.g. ready-to-use beverages, compositions are advantageous which are essentially free from, or comprise less than 5% by weight of, mono-saccharides. If monosaccharides are present, glucose galactose and tagatose each preferably account for less than 40 % by weight, more preferably less than 10 % by weight, even more preferably less than 1 % by weight of the mono-saccharides. In other applications such as ready-to-use snacks, however, inclusion of a carbohydrate source may be advantageous, preferably in an amount to provide 1 to 70 %, more preferably 25 % to 45 % of the energy on basis of the total energy of the composition.

Non-caloric sweeteners, flavourings and food-acceptable colourings may also be included.

A particularly advantageous embodiment comprises a liquid composition such as a ready-to-use beverage based on fruit juice, vegetable juice, water, isotonic drinks, carbonated flavoured drinks, soft drinks, teas, coffees, dairy products, meat and/or vegetable soups or mixtures thereof, which may be supplemented with minerals, vitamins and/or carbonic acid, if desired. Beverages comprising fruit or vegetable juices provide additionally the advantage of comprising vitamins, minerals or even enzymes and provide an advantageous complementation of a nutritional composition according to the present invention. In particular, juices such as orange, apple, pineapple, grapefruit, lemon, lime, mango, passion fruit, elderberries, cranberries, currants, grape, tomato, carrot or combinations thereof may form the basis for a ready-to-use beverage.

A liquid composition may comprise from 11 to 97 % by weight, preferably from 21 to 80 % by weight, most preferably from 61 to 75 % by weight, of any of the before-mentioned juices, beverages, water or mixtures thereof, and from 3 to 89 % by weight, preferably from 20 to 79 % by weight, most preferably from 25 to 39 % by weight, of a composition according to the present invention, on basis of the total weight of the liquid composition.

A liquid composition will preferably include 1 to 20 % by weight intact whey proteins, more preferably 5 to 12 % by weight intact whey proteins.

Advantageously, a beverage according to the present invention delivers 1 to 150 kcal, preferably 21 to 100 kcal, more preferably 31 to 50 kcal per 100 g of fluid preparation. For example, a beverage accompanying a standard meal may e.g. provide per dosage (i.e. per standard meal) 40 to 60 g of intact whey proteins.

Of course, consumers may also prepare such a beverage by mixing a composition according to the present invention (e.g. according to instructions on the package) with a beverage of their choice.

Alternatively, a food product may be enriched with a composition according to the present invention. For example, a fermented milk, a yoghurt, a fresh cheese, a renneted milk, a confectionery bar, breakfast cereal flakes or bars, a drink, milk powder, soy-based product, non-milk fermented product or a nutritional supplement for clinical nutrition. Then, the amount of the composition added is preferably, at least 0.5 % by weight, more preferably 11 to 40 % by weight, on basis of the total weight of the food product.

Food products or beverages as detailed above, provide the advantage that they may be consumed shortly before, during, or shortly after a meal by a person, in particular from a person suffering from Type 2 diabetes, and permit an easy solution for enhancing post-prandial insulinemia. Thus, compositions according to the present invention may be helpful in significantly increasing the quality of life of large groups of the population.

A composition according to the present invention may also be used for the preparation of an enteral nutritional formula, in particular for patients suffering from muscle depletion or for supporting muscle maintenance.

Compositions according to the present invention may be designed both for human consumption and for consumption by a companion animal, in particular for dogs and cats.

All before-mentioned products according to the present invention provide the advantage that they may be expected to be highly accepted by the consumers as they are formulated on basis of well-known nutritional components, which proved to be essentially free of undesired side-effects. Moreover, compositions according to the present invention are essentially free of unpleasant tastes and may be regularly, e.g. daily consumed.

According to another aspect, the invention also provides a method for treating or preventing metabolic dysfunctions and/or improving conditions associated with Type 2 diabetes mellitus or insulin resistance which comprises administering an effective amount intact whey proteins.

The following examples are given by way of illustration only and should not be construed as limiting the subject-matter of the present application.

Example 1

Influence of protein on the insulin response

This example demonstrates the effect of the protein digestion rate and compares the effects of intact whey and casein proteins.

The following abbreviations are used:

C_{max} is the maximal plasma concentration of a compound, T_{max} is time to achieve C_{max}, AUC is the area under the plasma concentration curve versus time, and p is the treatment effect.

The following regimens were administered:

- Treatment with sweet whey protein isolate (Lacprodan DI-9224, Arla Foods, Denmark), a protein which is providing for a rapid digestion rate (labelled with code "S1" below);
- Treatment with micellar casein (Promilk 852B, Ingredia Lactoprosperité AG, Switzerland), a comparative protein providing an essentially slow protein digestion rate (labelled with code "S2" below);

Each regimen was a protein powder and was reconstituted in a liquid form by mixing 100 g of protein powder with 900 g water. This solution delivers 40 kcal and 10 g protein per 100 g. Formula dosage depends on patient weight: 7 g liquid formula/kg body weight (BW) (0.7 g protein/kg BW). It was administrated as part of a test meal (6 kcal/kg BW; 38% carbohydrates, 15% lipids and 47% proteins). In order to obtain a high insulin response allowing for product discrimination, the protein solutions were ingested with carbohydrates and lipids (bread and chocolate spread) and the amount of protein was relatively high (around 2/3 of the protein daily requirements).

Study setup

The study was designed as a double-blind, single center, exploratory, randomized and controlled cross-over clinical trial. It has been carried out at the Centro Antidiabetico, Azienda Ospedaliera de Padova, Italy. The subjects were Type 2 diabetic patients. The treatments were blind to patients and to the study staff. Patients received once each treatment with a wash-out period of at least 2 weeks between treatments.

The study was performed on Type 2 diabetic patients of both sexes aged between 31 to 65 years from the outpatient diabetic subjects scheduled to be regularly visited at the Centro Antidiabetico of the Azienda Ospedialera of Padova.

The inclusion criteria were: more than 3 years of disease; defective endogenous insulin

secretion [C-peptide response peak after iv glucagon ≤ 3 mg/ml]; age: 30 - 65 years; 18 < BMI (Body Mass Index) < 30 kg / m^2; having obtained his/her informed consent; diet and/or OHA (oral hypoglycaemic agent)-treated.

The exclusion criteria were: treated with insulin; patients with moderate to severe kidney or liver insufficiency, respiratory or cardiac failure, endocrinopathies other than diabetes, and major diseases of the GI tract causing malabsorption; patients who cannot be expected to comply with the treatment; currently participating or having participated in another clinical trial during the last 3 months prior to the beginning of this study.

Each subject consumed both test treatments once in random order. The test period lasted one day. Twenty-four hours before and during test day, the patients had to interrupt the OHA therapy. At test day, the patients came to the hospital after overnight fasting. After placing an indwelling catheter in the patient arm, and taking two basal blood samples, patients consumed the test meal. The test meal included a liquid formula containing one of the treatments. Blood sampling was done at -10, 0, 10, 20, 30, 60, 90, 120, 150 and 180 minutes of the test meal intake.

Data collection, management and validation

The following data were collected:

Test periods

- Blood parameters: for each test period, the blood was collected for 190 minutes (2 samples before and 8 samples after the test meal). Then, the following plasmatic parameters were measured:
  - *amino acids:* taurine, aspartate, threonine, serine, asparagine, glutamate, glutamine, proline, glycine, alanine, citrulline, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, ornithine, lysine, histidine and arginine.
  - *hormones and metabolites:* proinsulin, insulin, GLP-1, GIP, glucagon, glucose, C-peptide, triglycerides and total cholesterol.

- Anthropometric measures: weight and height immediately before the test meal.

Statistical methods

Statistical analyses planned in the protocol

The primary and secondary outcomes were analyzed by using a linear mixed-effect model with the two treatments and sex as fixed effects and subject as random effect. The results include: mean ± standard deviation and 95% confidence interval for mean difference. The rejection level in statistical tests was equal to 5% (p=0.05). The statistical analyses were done using SAS software (version 8.2).

Calculations of the kinetic parameters

AUC is the area under the plasma concentration curve versus time. It is calculated by the trapezoidal rule as follows:

$$AUC = \frac{1}{2}\sum_{i=1}^{n-1} (T_{i+1} - T_i)\,(C_{i+1} + C_i - 2B)$$

where T_i is the i^{th} time value, C_i is the i^{th} concentration value, n is the number of time values and B is the baseline value. The kinetic parameters were calculated using NCSS2000 software.

Results

Compliance

For each test meal, the average amounts of bread, chocolate spread and protein drink are 27.46 ± 3.97 g of bread, 20.85 ± 3.00 g of chocolate and 487 ± 70 g of protein drink.

Primary outcome: Plasmatic C-peptide

The kinetic parameters of C-peptide [$AUC_{(0\text{-}180min)}$, C_{max} and T_{max}) from the treatments are summarized in Tables 2.

*Table 1-a: $AUC_{(0-180min)}$ of C-peptide [(ng/ml)*min]*

DESCRIPTIVE STATISTICS	TREATMENT	
	"Whey protein" S1	"Casein" S2
N	12	11
Mean	672	572
± SD	268	213
95% CI	[502; 843]	[428; 715]
Minimum	385	291
Median	570	552
Maximum	1213	901
[S1 – S2]	122 ± 82 (SE= 35) [50; 193]	

Mean ± standard deviation; [ ]: 95% confidence interval for mean; SE: Standard error of the mean; SD (standard deviation); CI (confidence interval).

The bioavailability [$AUC_{(0-180min)}$] is significantly different between the treatments. The AUC of treatment [S2; "casein"] is significantly lower than [S1; "whey protein"] (p= 0.002).

Table 1-b: $C_{max.}$ of C-peptide [ng/ml]

DESCRIPTIVE STATISTICS	TREATMENT	
	"Whey protein" S1	"Casein" S2
N	12	11
Mean	5.11	4.33
± SD	2.23	1.47
95% CI	[3.70; 6.52]	[3.34; 5.32]
Minimum	2.76	1.88
Median	4.90	4.36
Maximum	10.40	6.20
[S1 – S2]	0.94 ± 0.84 (SE= 0.35) [0.21; 1.67]	

Mean ± standard deviation; [ ]: 95% confidence interval for mean; SE: Standard error of the mean; SD (standard deviation); CI (confidence interval).

C_{max}, i.e. the maximal plasma concentration of the C-peptide, is significantly different between the treatments. The C_{max} of treatment [S2; "casein"] is significantly lower than [S1; "whey protein"] (p= 0.015).

Table 1-c: T_{max} to reach the C_{max} of C-peptide [min.]

DESCRIPTIVE STATISTICS	TREATMENT	
	"Whey protein" S1	"Casein" S2
N	12	11
Mean	117	129
± SD	24	54
95% CI	[102; 132]	[93; 165]
Minimum	80	15
Median	127	138
Maximum	145	206
[S1 – S2]	-10 ± 34 (SE= 14) [-40; 19]	

Mean ± standard deviation; [ ]: 95% confidence interval for mean; SE: Standard error of the mean ; **SD (standard deviation); CI (confidence interval).**

T_{max}, i.e. the time to reach the maximal plasma concentration of the C-peptide C_{max} is not significantly different between the treatments (p= 0.43).

Secondary outcomes : Hormones and metabolites

Proinsulin

Proinsulin is a precursor of insulin, which is obtained after cleavage of the C-peptide

and after formation of S-S-bridges. The kinetic parameters of proinsulin ($AUC_{(0\text{-}180min)}$, C_{max} and T_{max}) for the treatments are summarized in Table 2.

Table 2: Kinetic parameters of proinsulin

KINETIC PARAMETERS	DIFFERENCE BETWEEN THE TREATMENTS [S1 – S2]	TREATMENT EFFECT
AUC [(pM) * min]	752 ± 1189 [-329; 1834]	p > 0.05
C_{max} (pM)	8 ± 7 [1; 15]	p > 0.05
T_{max} (min)	-26 ± 26 [-49; -3]	p > 0.05

Mean ± standard deviation; [ ] : 95% confidence interval for mean difference.

Between 1 to 180 minutes, the plasmatic amount of proinsulin (bioavailability) and C_{max} of proinsulin with the treatment [S2; "casein"] does not significantly differ from [S1; "whey protein"]. T_{max} of proinsulin is not significantly different between the treatments (p= 0.07).

Insulin

The kinetic parameters of insulin ($AUC_{(0\text{-}180min)}$, C_{max} and T_{max}) from the treatments are summarized in Table 3.

Table 3: Kinetic parameters of insulin

KINETIC PARAMETERS	DIFFERENCE BETWEEN THE TREATMENTS [S1 – S2]	TREATMENT EFFECT
AUC [(μU/ml) * min]	1052 ± 1139 [56; 2049]	p > 0.05
C_{max} (μU/ml)	13 ± 12 [3; 24]	p = 0.014
T_{max} (min)	-7 ± 37 [-40; 25]	p > 0.05

Mean ± standard deviation; [ ] : 95% confidence interval for mean difference.

Between 0 and 180 minutes, the plasmatic amount of insulin (bioavailability) with the treatment [S2; "casein"] is not significantly different from [S1; "whey protein"]. C_{max} of

insulin with the treatment [S2; "casein"] is significantly lower than with the treatment [S1; "whey protein"] (p= 0.014). T_{max} of insulin is not significantly different between the treatments (p= 0.86).

Glucagon

Glucagon is a polypeptide hormone formed in the pancreas. The kinetic parameters of glucagon ($AUC_{(0\text{-}180min)}$, C_{max} and T_{max}) from the treatments are summarized in Table 4.

Table 4: Kinetic parameters of glucagon

KINETIC PARAMETERS	DIFFERENCE BETWEEN THE TREATMENTS [S1 – S2]	TREATMENT EFFECT
AUC [(µg/L) * min]	3955 ± 6311 [-1473; 9382]	p >0.05
C_{max} (µg/L)	78 ± 84 [4; 152]	p >0.05
T_{max} (min)	15 ± 24 [-5; 36]	p >0.05

Mean ± standard deviation; [ ] : 95% confidence interval for mean difference.

Between 0 and 180 minutes, the plasmatic amount of glucagon (bioavailability) with the treatments [S1] and [S2] is not significantly different (p= 0.13). C_{max} of glucagon with the treatments [S1] and [S2] is not significantly different (p= 0.10). T_{max} of glucagon is not significantly different between the treatments (p= 0.29).

As becomes obvious from the results above, the treatment with whey protein does not have a significant influence with respect to glucagon, a hormone which could give rise to an undesired blood sugar increase.

Glucose

The kinetic parameters of glucose ($AUC_{(0\text{-}180min)}$, C_{max} and T_{max}) from the treatments are summarized in Table 5.

Table 5: Kinetic parameters of glucose

KINETIC PARAMETERS	DIFFERENCE BETWEEN THE TREATMENTS [S1 – S2]	TREATMENT EFFECT
AUC [(mg/dl) * min]	-1447 ± 3533 [-4487; 1592]	p >0.05
C_{max} (mg/dl)	-7 ± 18 [-23; 9]	p >0.05
T_{max} (min)	1 ± 31 [-26; 29]	p >0.05

Mean ± standard deviation; [ ] : 95% confidence interval for mean difference.

Between 0 and 180 minutes, neither the plasmatic amount of glucose (bioavailability) nor the C_{max} of glucose nor the T_{max} of glucose is significantly different between the treatments (p= 0.66).

GIP (Gastric Inhibitory Peptide)

GIP (Gastric Inhibitory Peptide) is a gastrointestinal hormone which inhibits the liberation of insulin. The kinetic parameters of GIP ($AUC_{(0\text{-}150min)}$, C_{max} and T_{max}) from the three treatments are summarized in Table 6.

Table 6: Kinetic parameters of GIP

KINETIC PARAMETERS	DIFFERENCE BETWEEN THE TREATMENTS [S1 – S2]	TREATMENT EFFECT
AUC [(pM) * min]	343 ± 1260 [-442; 1128]	p >0.05
C_{max} (pM)	0 ± 15 [-10; 9]	p >0.05
T_{max} (min)	-10 ± 52 [-42; 22]	p >0.05

Mean ± standard deviation; [ ] : 95% confidence interval for mean difference.

Between 0 and 150 minutes, neither the plasmatic amount of GIP (bioavailability) nor the C_{max} of GIP nor the T_{max} of GIP is significantly different between the treatments (p= 0.61).

GLP-1 (Glucagon Like Polypeptide)

GLP-1 is a gastrointestinal hormone that has been reported to stimulate the release of insulin by the pancreatic beta-cells.

The kinetic parameters of GLP-1 ($AUC_{(0\text{-}150min)}$, C_{max} and T_{max}) from the treatments are summarized in Table 7

Table 7: Kinetic parameters of GLP-1

KINETIC PARAMETERS	DIFFERENCE BETWEEN THE TREATMENTS [S1 – S2]	TREATMENT EFFECT
AUC [(pM) * min]	234 ± 399 [-23; 490]	p = 0.05
C_{max} (pM)	4 ± 8 [-1; 8]	p >0.05
T_{max} (min)	4 ± 43 [-23; 32]	p >0.05

Mean ± standard deviation; [ ] : 95% confidence interval for mean difference.

Between 0 and 150 minutes, the plasmatic amount of GLP-1 (bioavailability) is not significantly different with the treatments [S1] and [S2] (p= 0.05). C_{max} of GLP-1 is not significantly different between the treatments (p= 0.09). T_{max} of GLP-1 is not significantly different between the treatments (p= 0.32).

Total cholesterol

The kinetic parameters of total cholesterol ($AUC_{(0\text{-}180min)}$, C_{max} and T_{max}) from the treatments are summarized in Table 8.

Table 8: Kinetic parameters of total cholesterol

KINETIC PARAMETERS	DIFFERENCE BETWEEN THE TREATMENTS [S1 – S2]	TREATMENT EFFECT
AUC [(mg/dl) * min]	-1273 ± 3318 [-4127; 1581]	p >0.05
C_{max} (mg/dl)	-6.70 ± 18 [-22; 9]	p >0.05
T_{max} (min)	11 ± 42 [-26; 47]	p >0.05

Mean ± standard deviation; [ ] : 95% confidence interval for mean difference.

Between 0 and 180 minutes, the plasmatic amount of total cholesterol (bioavailability) is not significantly different between the treatments [S1] and [S2] (p= 0.62). C_{max} of cholesterol is not significantly different between the treatments [S1] and [S2] (p= 0.59). T_{max} of cholesterol is not significantly different between the treatments (p= 0.33).

This example shows that administration of intact whey proteins is not associated with a negative effect with respect to the cholesterol level in blood.

Triglycerides

The kinetic parameters of triglycerides ($AUC_{(0\text{-}180min)}$, C_{max} and T_{max}) from the treatments are summarized in Table 9.

Table 9: Kinetic parameters of triglycerides

KINETIC PARAMETERS	DIFFERENCE BETWEEN THE TREATMENTS [S1 – S2]	TREATMENT EFFECT
AUC [(mg/dl) * min]	-454 ± 3727 [-3660; 2753]	p >0.05
C_{max} (mg/dl)	-13 ± 27 [-37; 11]	p >0.05
T_{max} (min)	-19 ± 53 [-65; 27]	p >0.05

Mean ± standard deviation; [ ] : 95% confidence interval for mean difference.

Between 0 and 180 minutes, the plasmatic amount of triglycerides (bioavailability) is

not significantly different between the treatments [S1] and [S2] (p= 0.96). C_{max} of triglycerides is not significantly different with the treatments [S1] and [S2] (p= 0.36). T_{max} of triglycerides is not significantly different between the treatments (p= 0.69).

This example shows that administration of intact whey proteins is not associated with a negative effect with respect to the trigyceride level in blood.

Example 2

Composition for use in the present invention

An enteral composition containing whey protein with an energy density of 4.6 KJ/ml and 9% (p/v) proteins was prepared from sweet whey protein isolate. 500 g sweet whey protein isolate, 250 g maltodextrin, 20 g non-caloric sweetener, 20.3 g tri-K citrate H2O, 9.2 g MgCl2.6H2O and 5.8 g NaCl were dispersed in 4.7 Kg of demineralised water at a temperature of about 50-55°C. The pH was adjusted to 6.8 after which 300 g fatty phase were introduced the total weight of the dispersion being 5 Kg. The dispersion was homogenised and sterilised. The resulting composition had an agreeable sweet taste.

It should be understood that various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present invention and without diminishing its attendant advantages. It is therefore intended that such changes and modifications will be covered by the appended claims.

Claims

1. Use of a composition comprising intact whey proteins for the preparation of a nutritional and/or a pharmaceutical composition for treating, preventing and/or improving metabolic dysfunctions and conditions associated with Type 2 diabetes mellitus.

2. The use according to claim 1, wherein the whey protein is sweet whey protein.

3. The use according to claim 1 or 2, wherein the amount of protein in the composition is in the range of from 1 to 90 % by weight, preferably from 5 to 70 % by weight, more preferably 11 to 50 % by weight, even more preferably 21 to 40 % by weight, most preferred about 25 to 35 % by weight, based on the total dry weight of the composition.

4. The use according to any preceding claim, for enhancing post-prandial insulinemia, stimulating insulin production, and/or decreasing blood glucose levels.

5. A method for treating, preventing and/or improving metabolic dysfunctions or conditions associated with Type 2 diabetes mellitus which comprises administering an effective amount of a composition containing intact whey proteins.

6. The method of claim 6 in which intact whey proteins are administered in an amount of 0.1 to 0.8g intact whey proteins per kg body weight.